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Research Article

Two new acremonium-like species, *Paragibellulopsis sinensis* sp. nov. and *Phialoparvum sinense* sp. nov. (Sordariomycetes, Ascomycota) from China

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Abstract

Paragibellulopsis and *Phialoparvum*, both erected in 2018, currently comprise merely one and three validly published species, respectively. The identification of two new species, *Paragibellulopsis sinensis* and *Phialoparvum sinense*, was achieved by analyzing morphological characteristics and phylogenetic data obtained from four molecular markers (ITS, LSU, *rpb2*, and *tef-1α*). Morphologically, *Pa. sinensis* possessed short conidiogenous cells and aseptate and smaller conidia. On the other hand, *Ph. sinense* had obovate conidia and longer phialides. Phylogenetic analysis revealed that *Pa. sinensis* formed an independent branch as a sister species to *Pa. chrysanthemi*, whereas *Ph. sinense* clustered in a monophyletic clade with a stable topology. The maximum likelihood bootstrap ratio and the Bayesian inference posterior probability provided robust statistical evidence, indicating the presence of two novel species within the genera of *Paragibellulopsis* and *Phialoparvum*. The present study contributes to the discovery of species diversity in *Paragibellulopsis* and *Phialoparvum*.

Key words: Acremonium-like species, fungal classification, new taxa, plectosphaerellaceous fungi, urban soil

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Introduction

Acremonium has long been recognized as one of the most simply structured fungi of all filamentous asexual fungi (Hou et al. 2023) and referred to the family Bionectriaceae (Hyde et al. 2024). The main characteristics of *Acremonium* are the production of septate hyphae giving rise to narrow, tapered, mostly lateral phialides with unicellular conidia arranged in mucoid heads or unconnected chains, and differentiated conidiophores with or without verticillate branches may be observed in some species (Gams 1971, 1975; Domsch et al. 2007; Perdomo et al. 2011; Summerbell et al. 2011). Although the asexual morph of *Acremonium* is relatively plesiomorphic in morphology, the genus is heterogeneous due to the fact that numerous sexually reproducing ascomycetous genera with diverse morphologies possess acremonium-like asexual morphs.

Recently, Hou et al. (2023), through extensive taxonomic sampling within Hypocreales and multilocus phylogenetic analyses, further confirmed the highly polyphyletic nature of acremonium-like taxa and demonstrated that these taxa are widely distributed across multiple families, including Cephalothecaceae, Chrysonectriaceae, Clavicipitaceae, Cordycipitaceae, Myrotheciomycetaceae, Nectriaceae, Neoacremoniaceae, Niessliaceae, Nothoacremoniaceae, Plectosphaerellaceae, Pseudoniessliaceae, and Valsonectriaceae. This finding further demonstrates that due to the morphological plasticity of acremonium-like species, clear taxonomic delineation of these fungi remains unattainable without DNA barcoding and phylogenetic analyses. Consequently, researchers must integrate DNA sequence data to substantiate morphological and ecological interpretations (Verkley et al. 2013; Chen et al. 2015; Crous et al. 2021).

Zare et al. (2007) established the family Plectosphaerellaceae based on its close relationships to Glomerellaceae within Glomerellales when revising verticillium-like taxa. Giraldo and Crous (2019) comprehensively clarified the systematic status of members of Plectosphaerellaceae based on multi-locus phylogenetic analyses and morphological evidence. Recently, Crous et al. (2023b) proposed the reduction of Plectosphaerellaceae under Trichosphaeriaceae and its elevation to the order Trichosphaeriales, based on a single fungal isolate. However, the type species of two recently described genera, *Parafuscohypha* (only *Para. proliferate*, ex-type CBS 308.74) and *Truncatascus* (only *T. microsporus*, ex-type GZAAS 19-1744), exhibited almost identical ITS and *tef-1α* sequences to a putative *Trichosphaeria pilosa* isolate (CBS 149698, which was designated epitype), suggesting these genera may represent synonyms unless the results were due to contamination (Yeh et al. 2024). These taxonomic changes, based on a single ascomata isolate, are particularly problematic due to potential misidentification or contamination (Crous et al. 2023b). Moreover, the designation of epitype under such circumstances proves superfluous, contravenes established taxonomic protocols, and potentially leads to misleading conclusions (as in *Trichosphaeria*) (Yeh et al. 2024). In light of these concerns, Giraldo et al. (2019) and Yeh et al. (2024) employ the term “plectosphaerellaceous fungi” to replace potentially ephemeral discrete taxonomic names.

In the present study, we aimed to isolate microfungi from urban soil samples in Beijing and Guizhou, China. This study has the following objectives: 1) to describe two novel acremonium-like species (affiliated with the genera *Paragibellulopsis* and *Phialoparvum*) through comprehensive morphological examinations and phylogenetic analyses of ITS, LSU, *rpb2*, and *tef-1α* sequence data; 2) to provide a checklist that includes substrate, availability of molecular data, morphological characteristics, and country of origin.

Materials and methods

Fungal isolation and morphological characterization

Samples were collected from the green belts of Guizhou Wildlife Park and Beijing Xishan National Forest Park on 17 July 2022 and 4 June 2024, respectively. The samples were collected 3–10 cm below the soil surface, were stored in Ziploc plastic bags, transported to the laboratory, and promptly processed. Fungi were isolated and purified using the method described by Zhang et al. (2023, 2024).

Colony characters and diameters were recorded from cultures grown on potato dextrose agar (PDA), synthetic low-nutrient agar (SNA), and oatmeal agar (OA) plates at 25 °C in the dark for 14 days. For light microscopic observations, slides were prepared from cultures grown on PDA, and the characterization and measurement of fungal microscopic characteristics were performed in 25% clear lactic acid. Other microscopic examinations followed procedures outlined by Senanayake et al. (2020). Fungal structures were photographed using a Nikon ECLIPSE Ni compound microscope fitted with a Nikon DS-Ri2 digital camera. Measurements for all structural components were made with Tarosoft Image FrameWork software (IFW).

Descriptions of novelties were deposited in MycoBank, and the type strains for the novel taxa represented by living cultures were deposited at the China General Microbiological Culture Collection Center (CGMCC), China. The holotype (dried cultures) of the novel species was deposited at the Fungarium (HMAS), Institute of Microbiology, Chinese Academy of Sciences (CAS). In addition, all living cultures were stored in a metabolically inactive state (i.e., kept in sterile 30% glycerol in a –80 °C freezer), which were deposited in the College of Eco-Environmental Engineering, Guizhou Minzu University, China.

DNA extraction, PCR amplification, and sequencing

The BioTeke Fungal Genomic DNA Extraction Kit (DP2032, BioTeke, Beijing, China) was used to extract genomic DNA from fungal hyphae according to the manufacturer's instructions. Four gene regions, internal transcribed spacers (ITS), large subunit rDNA (LSU), transcription elongation factor 1-alpha gene region (*tef-1α*), and RNA polymerase II subunit (*rpb2*), were amplified and sequenced using primers listed in Table 1. Polymerase chain reactions (PCR) were carried out in a 25 µL reaction volume, which contained 12.5 µL 2 × PCR Master Mix (Sangon Biotech, China), 8.5 µL ddH_2O, 1 µL of each primer, and 2 µL DNA template. All newly generated sequences have been deposited in the GenBank database (Table 2).

Phylogenetic analyses

Lasergene software (version 6.0, DNASTAR) was used to edit the ambiguous bases of the PCR amplicon sequences. Combined sequences of ITS, LSU, *rpb2*, and *tef-1α* were analyzed to infer the phylogenetic placement of *Pa. sinensis* and *Ph. sinense*. The sequences were aligned and adjusted by MAFFT v7.037

Table 1. Sequences of primers used in this study.

Gene/ loci	PCR primers (forward/reverse)	Primer sequence (5´-3´)	Reference
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)
LSU	LR0R	ACCCGCTGAACTTAAGC	Vilgalys and Hester (1990)
	LR5	ATCCTGAGGGAAACTTC	Vilgalys and Hester (1990)
tef-1α	EF1-983F	GCYCCYGGHCAYCGTGAYTTYAT	Rehner and Buckley (2005)
	EF1-2218R	ATGACACCRACRGCRACRGTYTG	Rehner and Buckley (2005)
rpb2	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	Liu et al. (1999)
	RPB2-7cR	CCCATRGCTTGYTTRCCCAT	Liu et al. (1999)

Table 2. Strains used in this study, with information on the GenBank accessions of the sequences.

Species	Strains	GenBank accession no.			
		ITS	LSU	*rpb2*	*tef-1α*
Acremoniisimulans hongheensis	HKAS 122669 T	OQ379005	OQ379416	OQ378988	OQ378995
Acremoniisimulans hongheensis	HKAS 122670	OQ379006	OQ379417	OQ378989	OQ378996
Acrostalagmus annulatus	CBS 121.84	LR026673	LR025802	LR026104	LR026374
Acrostalagmus luteoalbus	CBS 112.16	LR026668	LR025797	LR026101	LR026369
Allomusicillium domschii	CBS 764.69 T	OQ429497	OQ055408	OQ453889	OQ470787
Brunneochlamydosporium nepalense	CBS 971.72 IT	LR026684	LR025813	LR026112	LR026386
Brunneochlamydosporium terrestre	CBS 112777 T	LR026690	LR025819	LR026118	LR026392
Brunneomyces brunnescens	CBS 559.73 T	LN810520	HQ231966	LR026119	LN810534
Brunneomyces europaeus	CBS 652.96 T	LN810519	LN810512	LN810528	LN810538
Chlamydosporiella restricta	CBS 178.40 T	LR026693	LR025822	LR026122	LR026395
Chlamydosporiella restricta	CBS 716.88	LR026696	LR025825	LR026125	LR026398
Chordomyces albus	CBS 987.87 T	DQ825970	JX158444	JX158466	JX158400
Chordomyces antarcticus	CBS 120045 T	KJ443241	KJ443109	KJ443157	KJ443197
Furcasterigmium furcatum	CBS 122.42 T	LR026709	LR025838	LR026133	LR026408
Furcasterigmium furcatum	CBS 116548	LR026712	LR025842	LR026134	LR026409
Fuscohypha expansa	CBS 103.95	LR026714	LR025844	NA	LR026411
Fuscohypha expansa	CBS 418.89 T	LR026715	LR025845	LR026136	LR026412
Gibellulopsis nigrescens	CBS 120949 NT	LR026738	LR025868	LR026149	LR026429
Gibellulopsis serrae	CBS 290.30 T	LR026742	LR025872	NA	LR026433
Houtenomyces caricicola	CBS 149675 T	OQ628483	OQ629065	OQ627944	NA
Lectera humicola	IMI 265740 T	JQ647449	LR025896	LR026169	LR026457
Lectera phaseoli	IMI 366179 T	JQ693168	LR025898	LR026171	LR026459
Longitudinalis nabanheensis	KUMCC 16-0145 T	KY882037	NG_068250	NA	KY882040
Musicillium theobromae	CBS 968.72 NT	LR026773	LR025907	LR026178	LR026468
Musicillium tropicale	CBS 120009 T	LR026783	LR025917	LR026186	LR026477
Musidium stromaticum	CBS 135.74A	LR026787	LR025922	LR026189	LR026482
Musidium stromaticum	CBS 863.73 T	DQ825969	HQ232143	NA	LN810533
Nigrocephalum collariferum	CBS 124585	FJ765365	LR025928	LR026192	LR026485
Nigrocephalum collariferum	CBS 124586 T	FJ765367	LR025929	LR026193	LR026486
Parafuscohypha proliferata	CBS 308.74 T	OQ429771	OQ055669	OQ560705	OQ471098
Paragibellulopsis chrysanthemi	MAFF 242621 T	KC287235	KC287230	NA	KC287232
Paragibellulopsis chrysanthemi	MAFF 243429	KC287234	KC287229	NA	KC287231
Paragibellulopsis sinensis	**CGMCC 3.28460 = ZY 24.001 T**	**PQ536128**	**PQ536126**	**PQ540986**	**PQ540988**
Paragibellulopsis sinensis	**ZY 24.002**	**PQ536129**	**PQ536127**	**PQ540987**	**PQ540989**
Paramusicillium asperulatum	CBS 120158 T	LR026792	LR025930	LR026194	LR026487
Phaeochloridium taiwanense	BCRC FU31690 T	PP278369	NA	LC799521	LC799522
Phaeochloridium taiwanense	R. Kirschner 5374	PP278370	NA	LC799524	LC799523
Phialoparvum bifurcatum	CBS 299.70B T	LR026793	LR025931	LR026195	LR026488
Phialoparvum maaspleinense	CBS 145321 T	LR590190	LR590368	LR594791	NA
Phialoparvum rietveltiae	CBS 145322 T	LR590191	LR590367	LR594790	NA
Phialoparvum sinense	**CGMCC 3.27537 = ZY 22.078 T**	**PP769719**	**PP769727**	**PP779747**	**PP779739**
Phialoparvum sinense	**ZY 22.079**	**PP769720**	**PP769728**	**PP779748**	**PP779740**
Phialoparvum sinense	**ZY 22.080**	**PP769721**	**PP769729**	**PP779749**	**PP779741**
Phialoparvum sinense	**ZY 22.081**	**PP769722**	**PP769730**	**PP779750**	**PP779742**
Plectosphaerella cucumerina	CBS 137.37 T	LR026798	LR025936	LR026199	LR026493
Plectosphaerella nauculispora	CGMCC 3.19656 T	MK880441	MK880431	MK930460	MK930453
Sayamraella subulata	BCC 78964 T	LR026833	LR025971	LR026226	LR026531

Species	Strains	GenBank accession no.			
		ITS	LSU	*rpb2*	*tef-1α*
Sinochloridium bambusicola	CGMCC 3.20735 T	OL628215	NA	NA	NA
Sodiomyces alcalophilus	CBS 114.92 IT	JX158421	JX158443	JX158465	JX158399
Sodiomyces alkalinus	CBS 110278 T	NR_145378	JX158427	JX158449	JX158383
Stachylidium bicolor	CBS 121802 ET	LR026834	LR025972	NA	LR026532
Stachylidium pallidum	DAOMC 226658	LR026838	GU180651	LR026228	LR026534
Summerbellia oligotrophica	CBS 299.70G	LR026716	LR025846	NA	LR026413
Summerbellia oligotrophica	CBS 657.94 T	LR026719	LR025849	NA	NA
Theobromium fuscum	CBS 112271 T	LR026839	LR025976	LR026229	LR026535
Trichosphaeria pilosa	CBS 149698 ET	OQ990134	OQ990085	OQ989224	OQ989249
Truncatascus microsporus	GZAAS 19-1744 T	OR225088	OP099542	OR146950	NA
Truncatascus microsporus	GZAAS 21-2018	OR225089	OR209670	OR146951	NA
Verticillium alfalfae	CBS 130603 T	LR026851	LR025988	LR026236	LR026547
Verticillium nubilum	CBS 457.51 T	LR026937	LR026076	LR026282	LR026591
Xenoplectosphaerella clematidis	MFLUCC 17-2067 T	NR_172181	NG_071256	MT394722	MT394674
Monilochaetes infuscans	CBS 379.77	LR026764	GU180645	GU180658	LR026460
Monilochaetes infuscans	CBS 869.66	GU180626	GU180639	GU180657	LR026461

Notes: T: Ex-type; IT: Ex-isotype; NT: Ex-neotype; ET: Ex-epitype; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; HKAS: herbaria of the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; KUMCC: Kunming Institute of Botany Culture Collection, Kunming, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Ibaraki, Japan; BCRC: Bioresource Collection and Research Center, Taiwan, China; BCC: BIOTEC Culture Collection, Pathumthani, Thailand; DAOMC: Canadian Collection of Fungal Cultures, Ontario, Canadian; GZAAS: Guizhou Academy of Agriculture Sciences, Guizhou, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; Other acronyms represent personal collections; NA, not available. DNA sequences for the new isolates were in bold.

(Katoh and Standley 2013) and MEGA 6.06 (Tamura et al. 2013). The "Concatenate Sequence" function in the PhyloSuite platform version 1.2.3 (Xiang et al. 2023) was used for the concatenation of loci.

The best-fit substitution model was selected for the Bayesian analysis and the maximum likelihood analysis using the corrected Akaike Information Criterion (AICc) in the ModelFinder (Kalyaanamoorthy et al. 2017). The maximum likelihood analysis was implemented in IQ-TREE v1.6.11 (Nguyen et al. 2015) with 10,000 bootstrap tests, using the ultrafast algorithm (Minh et al. 2013), in the PhyloSuite v.1.2.3 (Xiang et al. 2023). MrBayes version 3.2 (Ronquist et al. 2012) was used for the BI analysis. The Markov Chain Monte Carlo (MCMC) method was used to perform 5×10^7 simulations with a sampling frequency of 10^3 generations and a 25% burn-in. After the analysis was finished, Tracer v1.5 (Drummond and Rambaut 2007) was used to determine burn-in and confirm that both runs had converged.

Results

Phylogenetic analyses

Based on the combined dataset of the ITS, LSU, *rpb2*, and *tef-1α* sequences, the phylogeny of *Pa. sinensis*, *Ph. sinense*, and its related taxa was studied. *Monilochaetes infuscans* (CBS 869.66 and CBS 379.77) were used as the outgroup for the phylogenetic analysis. The concatenated dataset included 63 taxa and consisted of 2,823 nucleotides (ITS, 499 bp; LSU, 795 bp; *rpb2*, 743 bp; and *tef-1α*, 786 bp) with inserted gaps. The phylogenetic tree of the multi-locus dataset revealed that all genera were grouped in distinct branches with robust support, indicating a sta-

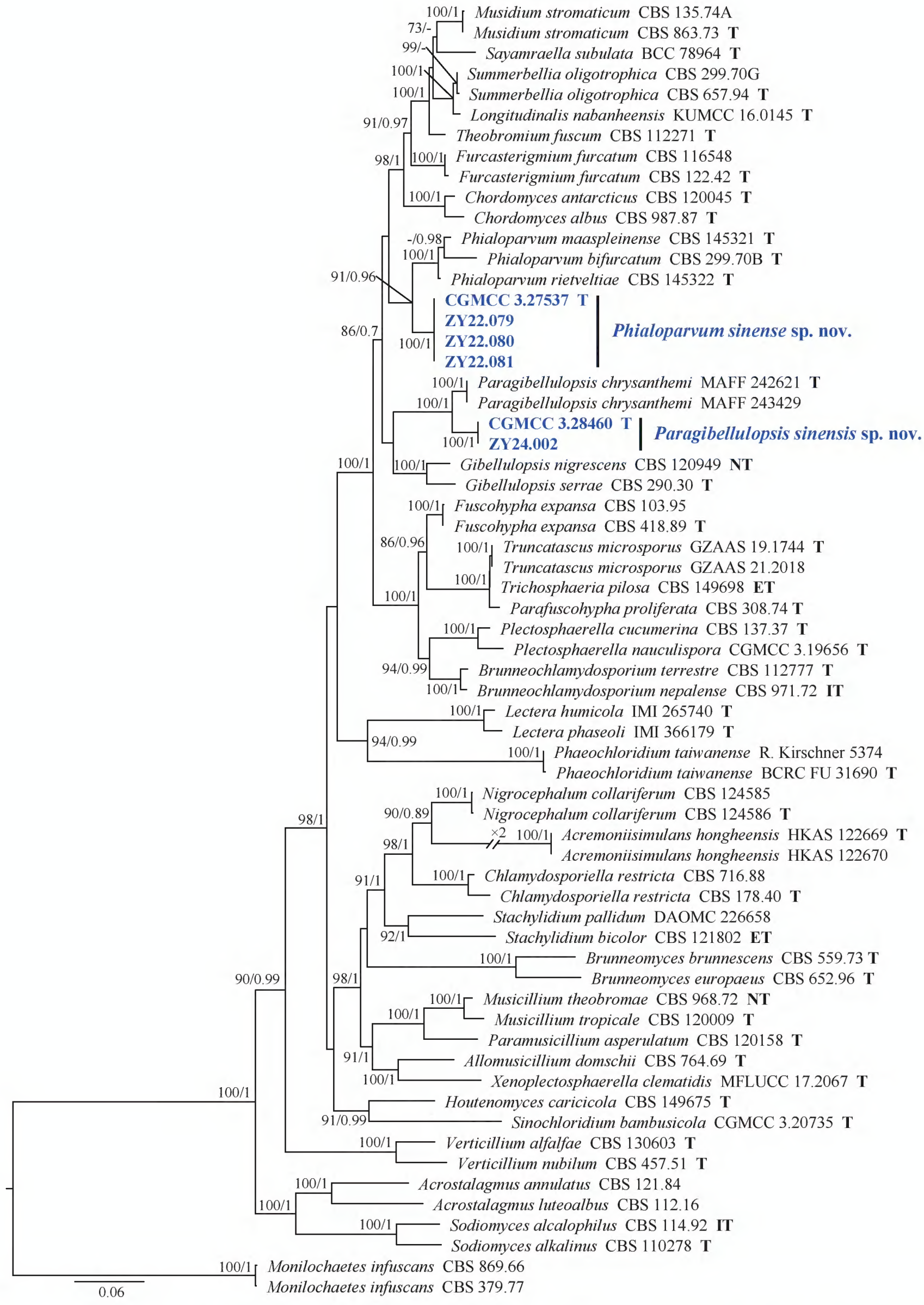
Musidium stromaticum CBS 135.74A
Musidium stromaticum CBS 863.73 T
Sayamraella subulata BCC 78964 T
Summerbellia oligotrophica CBS 299.70G
Summerbellia oligotrophica CBS 657.94 T
Longitudinalis nabanheensis KUMCC 16.0145 T
Theobromium fuscum CBS 112271 T
Furcasterigmium furcatum CBS 116548
Furcasterigmium furcatum CBS 122.42 T
Chordomyces antarcticus CBS 120045 T
Chordomyces albus CBS 987.87 T
Phialoparvum maaspleinense CBS 145321 T
Phialoparvum bifurcatum CBS 299.70B T
Phialoparvum rietveltiae CBS 145322 T
CGMCC 3.27537 T
ZY22.079
ZY22.080
ZY22.081
Phialoparvum sinense sp. nov.
Paragibellulopsis chrysanthemi MAFF 242621 T
Paragibellulopsis chrysanthemi MAFF 243429
CGMCC 3.28460 T
ZY24.002
Paragibellulopsis sinensis sp. nov.
Gibellulopsis nigrescens CBS 120949 NT
Gibellulopsis serrae CBS 290.30 T
Fuscohypha expansa CBS 103.95
Fuscohypha expansa CBS 418.89 T
Truncatascus microsporus GZAAS 19.1744 T
Truncatascus microsporus GZAAS 21.2018
Trichosphaeria pilosa CBS 149698 ET
Parafuscohypha proliferata CBS 308.74 T
Plectosphaerella cucumerina CBS 137.37 T
Plectosphaerella nauculispora CGMCC 3.19656 T
Brunneochlamydosporium terrestre CBS 112777 T
Brunneochlamydosporium nepalense CBS 971.72 IT
Lectera humicola IMI 265740 T
Lectera phaseoli IMI 366179 T
Phaeochloridium taiwanense R. Kirschner 5374
Phaeochloridium taiwanense BCRC FU 31690 T
Nigrocephalum collariferum CBS 124585
Nigrocephalum collariferum CBS 124586 T
×2
Acremoniisimulans hongheensis HKAS 122669 T
Acremoniisimulans hongheensis HKAS 122670
Chlamydosporiella restricta CBS 716.88
Chlamydosporiella restricta CBS 178.40 T
Stachylidium pallidum DAOMC 226658
Stachylidium bicolor CBS 121802 ET
Brunneomyces brunnescens CBS 559.73 T
Brunneomyces europaeus CBS 652.96 T
Musicillium theobromae CBS 968.72 NT
Musicillium tropicale CBS 120009 T
Paramusicillium asperulatum CBS 120158 T
Allomusicillium domschii CBS 764.69 T
Xenoplectosphaerella clematidis MFLUCC 17.2067 T
Houtenomyces caricicola CBS 149675 T
Sinochloridium bambusicola CGMCC 3.20735 T
Verticillium alfalfae CBS 130603 T
Verticillium nubilum CBS 457.51 T
Acrostalagmus annulatus CBS 121.84
Acrostalagmus luteoalbus CBS 112.16
Sodiomyces alcalophilus CBS 114.92 IT
Sodiomyces alkalinus CBS 110278 T
Monilochaetes infuscans CBS 869.66
Monilochaetes infuscans CBS 379.77
0.06

Figure 1. Phylogenetic tree inferred from a maximum likelihood analysis based on a concatenated alignment of ITS, LSU, *rpb2*, and *tef-1α* sequences of 63 strains representing plectosphaerellaceous fungi and outgroups. The ML bootstrap support values (MLBS) above 70% and Bayesian posterior probabilities (BPP) above 0.70 are given at the nodes (MLBS/BPP). Strains with special status are indicated with a superscript letter after the accession number (T: ex-type, ET: ex-epitype, IT: ex-isotype, NT: ex-neotype). The new species are printed in blue font. The tree is rooted to *Monilochaetes infuscans* CBS 869.66 and CBS 379.77.

ble topology (Fig. 1). The isolates CGMCC 3.28460 and ZY 24.002, collected and described in this study, formed a well-supported single branch. *Pa. sinensis* and *Pa. chrysanthemi* were identified as sister species, constituting an independent clade with BP and BPP values of 100% and 1, respectively, with a stable topology. In addition, CGMCC 3.27537, ZY 22.079, ZY 22.080, and ZY 22.081 clustered together (BP = 100%, BPP = 1) and were closely related to *Phialoparvum s. str.* (Fig. 1).

Taxonomy

***Paragibellulopsis sinensis* H. Pan & Zhi.Y. Zhang, sp. nov.**

MycoBank No: 856667

Fig. 2

Type. China • Beijing, Xishan National Forest Park, 39.97°N, 116.20°E, soil, 4 June 2024, Zhi-Yuan Zhang (holotype HMAS 353383, dried culture; culture ex-type CGMCC 3.28460, *ibid*., ZY 24.001).

Etymology. The epithet "sinensis" (Lat.) refers to China, where the species was collected.

Description. ***Culture characteristics*** (14 days at 25 °C): Colony on PDA 35–37 mm diam. white to grey, felty, margin fimbriate; reverse: white to grey. Colony on OA 44–46 mm diam. white to grey, flat, margin entire, aerial mycelia extremely sparse; reverse: white to grey. Colony on SNA 25–30 mm diam. white, floccose, margin irregular; reverse: white.

Mycelium consisting of branched, septate, hyaline, and smooth-walled hyphae, 1–3 µm wide. ***Conidiophores*** arising from submerged or superficial hyphae, erect or slanted, simple or poorly branched, often reduced to conidiogenous cells. ***Conidiogenous cells*** monophialidic, terminal, lateral, cylindrical, straight or curved, hyaline, smooth-walled, with funnel-shaped collarette and a distinct periclinal thickening at the conidiogenous locus, 20–39 µm long, 1.5–4 µm wide at the base. ***Conidia*** aggregated in small, slimy heads, hyaline, cylindrical with tapering ends, straight or slightly curved, smooth-walled, 1-celled, 5.5–9 × 2–2.5 µm (n = 50). ***Chlamydospores*** terminal, lateral, intercalary, single, or, in short, in chains, globose to ellipsoid, cylindrical, hyaline, smooth-walled, 6–10 × 5.5–7 µm (n = 50). ***Sexual morph*** unknown.

Geographical distribution. Beijing, China.

Additional material examined. China • Beijing, Xishan National Forest Park, 39.97°N, 116.20°E, soil, 4 June 2022, Xin Li, ZY 24.002.

Notes. Phylogenetic analysis showed that two new isolates (CGMCC 3.28460 and ZY 24.002) clustered in a single subclade with high supported value (100/1) and were nested in *Paragibellulopsis s. str.* (Fig. 1). Morphologically, *Pa. sinensis* differs from *Pa. chrysanthemi* by its short conidiogenous cells (20–39 µm in *Pa. sinensis* vs. 36–72 µm in *Pa. chrysanthemi*) and aseptate and smaller conidia (5.5–9 × 2–2.5 µm in *Pa. sinensis* vs. 12.5–15.5 × 2.7–3.7 µm in *Pa. chrysanthemi*) (Hirooka et al. 2014). In addition, *Pa. sinensis* can be distinguished from *Pa. chrysanthemi* by their low sequence similarities. In a comparison of ITS, LSU, and *tef-1α* nucleotides, *Pa. sinensis* (ex-type CGMCC 3.28460) has 97.2%, 98.5%, and 98.8% similarity in ITS (467/480 bp, four gaps), LSU (557/565 bp, no gap), and *tef-1α* (432/437 bp, no gap), which is different from *Pa. bifurcatum* (ex-type MAFF 242621).

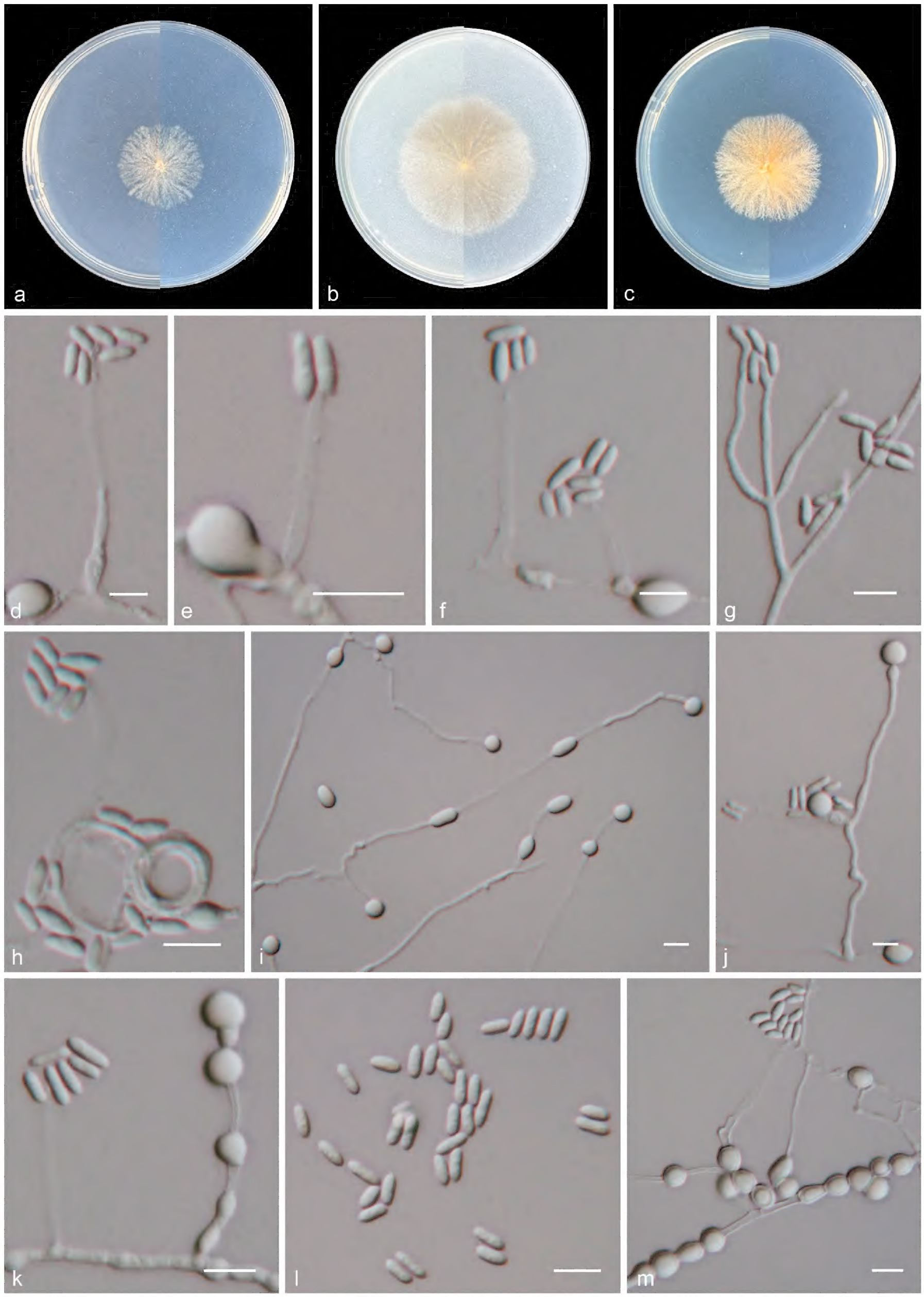

Figure 2. *Paragibellulopsis sinensis* (from holotype HMAS 353383) **a–c** upper and reverse views of cultures on PDA, OA, and SNA after 14 days at 25 °C **d–f** conidiogenous cells **g** conidiophores **h** hyphal coil and conidiogenous cells **i, m** chlamydospores **j, k** conidia and chlamydospores **l** conidia. Scale bars: 10 μm (**d–m**).

***Phialoparvum sinense* H. Pan & Zhi.Y. Zhang, sp. nov.**

MycoBank No: 853675

Fig. 3

Type. CHINA • Guizhou Province, Guiyang City, Xiuwen County, Zhazuo Town, Guizhou Wildlife Park, 26.85°N, 106.69°E, soil, 25 May 2022, Zhi-Yuan Zhang (holotype HMAS 353384, dried culture; culture ex-type CGMCC 3.27537, *ibid*., ZY 22.078).

Etymology. The epithet "*sinense*" (Lat.) refers to China, where the species was collected.

Description. ***Culture characteristics*** (14 days at 25 °C): Colony on PDA 67–69 mm diam. white aluminum to grey-white from center to margin, flocculent, subcircular, margin regular; reverse: tele grey to grey-white from center to margin. Colony on OA 58–60 mm diam. white, flocculent, subcircular, margin regular; reverse: white. Colony on SNA 25–27 mm diam. white, flattened, hyphae sparse; reverse: white.

Mycelium consisting of branched, septate, hyaline, and smooth-walled hyphae, 1–3 µm wide. ***Conidiophores*** solitary, erect, arising directly from vegetative hyphae or ropes of hyphae, unbranched or poorly branched. ***Phialides*** lateral, terminal, subulate, hyaline, smooth-walled, 8.5–50.5 × 1–3 µm, with cylindrical collarette and conspicuous periclinal thickening at the conidiogenous locus; adelophialides sometimes present, up to 4 µm long; polyphialides with two conidiogenous loci are occasionally present. ***Conidia*** arranged in slimy heads, obovate with slightly obtuse base, sometimes cylindrical with slightly obtuse base, 1-celled, hyaline, smooth-walled, 2–6.5 × 1–3 µm (av. 4.5 × 2, n = 50). ***Sexual morph*** undetermined.

Geographical distribution. Guizhou Province, China.

Additional material examined. CHINA • Guizhou Province, Guiyang City, Xiuwen County, Zhazuo Town, Guizhou Wildlife Park, 26.85°N, 106.69°E, soil, 25 May 2022, Z.Y. Zhang, ZY 22.079, *ibid*., ZY 22.080, and ZY 22.081.

Notes. Phylogenetic analysis showed that four new isolates (CGMCC 3.27537, ZY 22.079, ZY 22.080, and ZY 22.081) clustered in a single subclade with high support values (100/1) and were nested in *Phialoparvum s. str.* (Fig. 1). Morphologically, *Ph. sinense* differs from other species in the genus *Phialoparvum* in that it produces obovate conidia and longer phialides (Giraldo and Crous 2019; Giraldo et al. 2019). In addition, they can be distinguished by their low sequence similarities. In a comparison of ITS, LSU, *rpb2*, and *tef-1α* nucleotides, *Ph. sinense* (ex-type CGMCC 3.27537) has 95.3%, 97.2%, 93.5%, and 96.3% similarity in ITS (469/492 bp, seven gaps), LSU (773/795 bp, four gaps), *rpb2* (262/280 bp, no gap), and *tef-1α* (758/787 bp, no gap), which is different from *Ph. bifurcatum* (ex-type CBS 299.70B). In a comparison of ITS, LSU, and *rpb2* nucleotides, *Ph. sinense* (CGMCC 3.27537) has 96.5%, 98.1%, and 91.9% similarity in ITS (453/469 bp, four gaps), LSU (778/793 bp, no gap), and *rpb2* (751/817 bp, no gap), which is different from *Ph. maaspleinense* (ex-type CBS 145321). In a comparison of ITS, LSU, and *rpb2* nucleotides, *Ph. sinense* (CGMCC 3.27537) has 96.6%, 98.3%, and 92.7% similarity in ITS (483/500 bp, five gaps), LSU (780/793 bp, no gap), and *rpb2* (694/748 bp, no gap), which is different from *Ph. rietveltiae* (ex-type CBS 145322).

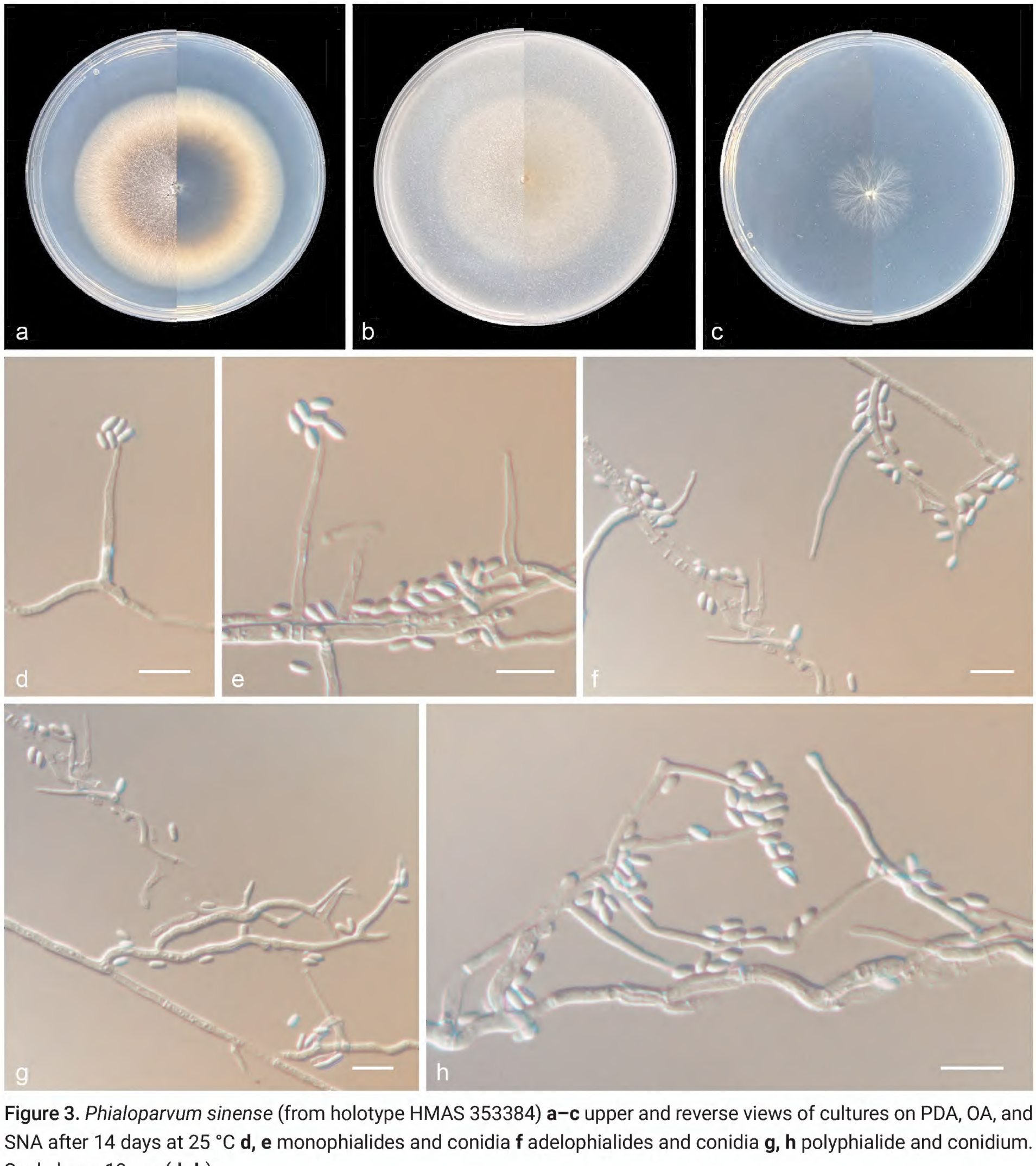

Figure 3. *Phialoparvum sinense* (from holotype HMAS 353384) **a–c** upper and reverse views of cultures on PDA, OA, and SNA after 14 days at 25 °C **d, e** monophialides and conidia **f** adelophialides and conidia **g, h** polyphialide and conidium. Scale bars: 10 μm (**d–h**).

Key to the genus *Phialoparvum*

1	Phialides subulate	**2**
–	Phialides subulate to ampulliform	**3**
2	Conidia cylindrical, occasionally with one or two guttules	***P. bifurcatum***
–	Conidia cylindrical, with slightly pointed ends	***P. rietveltiae***
3	Conidia cylindrical	***P. maaspleinense***
–	Conidia obovate	***P. sinense***

Discussion

Currently, the plectosphaerellaceous fungi comprise more than 20 genera (Hyde et al. 2017; Tibpromma et al. 2018; Giraldo and Crous 2019; Phukhamsakda et al. 2020; Crous et al. 2023a; Hou et al. 2023). They exhibited a wide distribution and were commonly observed on various substrates or hosts, including soil, plants, fungi, humans, decayed wood, bark, and leaves (Hyde et al. 2014; Giraldo and Crous 2019). While conducting taxonomic studies of genera within Plectosphaerellaceae based on multi-locus phylogenetic and morphological evidence, Giraldo and Crous (2019) transferred *Gibellulopsis chrysanthemi* from *Gibellulopsis* and established it as the type species of the newly erected genus *Paragibellulopsis*. To date, *Paragibellulopsis* includes only one species (*Pa. chrysanthemi*), which was isolated from rotten leaves of *Chrysanthemum coronarium* var. *spatiosum* (Hirooka et al. 2014). The novel species *Pa. sinensis* proposed in this study was isolated from soil, which expands our understanding of the trophic modes within this genus. Furthermore, Giraldo and Crous (2019) established the genus *Phialoparvum* based on *Ph. bifurcatum* as the type species, which was isolated from soil. Subsequently, two other valid species of the genus *Phialoparvum* (*Ph. maaspleinense* and *Ph. rietveltiae*) were reported, which were isolated from Dutch garden soil (Giraldo et al. 2019). All three species were isolated from Europe, whereas *Ph. sinense* was obtained from China, suggesting this genus may have a wide distribution. This study enhances our understanding of species diversity and geographical distribution within these two genera.

The macroscopic and microscopic morphology of most species in the plectosphaerellaceous fungi are quite similar, and it is difficult to distinguish specific species based on only morphological features. Thus, it is often necessary to combine morphological and molecular data for species identification. According to the new species delimitation criteria proposed by Jeewon and Hyde (2016), phylogenetic analyses should incorporate the ITS regions and at least one protein gene, with a minimum of >1.5% nucleotide differences in the ITS regions between closely related species. In this study, two novel species, *Pa. sinensis* and *Ph. sinense*, were identified and characterized through meticulous morphological examination, rigorous phylogenetic analyses, and nucleotide divergence assessments among closely related species.

It was observed that a prominent characteristic of species within the *Paragibellulopsis* genus was the monophialidic of conidiogenous cells, wherein conidia typically produced in slimy heads, cylindrical, straight, or slightly curved (Hirooka et al. 2014; Giraldo and Crous 2019). The primary distinguishing feature of *Phialoparvum* species lies in phialides enteroblastic, mono- and polyphialidic; conidia cylindrical, 1-celled, arranged in slimy heads (Giraldo and Crous 2019). The conidiogenous cells of *Pa. sinensis* collected in this study were monophialidic; the conidia aggregated in small, slimy heads, cylindrical, straight, or slightly curved. These characteristics aligned closely with the primary identification features described for *Paragibellulopsis* species by Giraldo and Crous (2019). However, *Pa. sinensis* differs primarily from *Pa. chrysanthemi* in producing aseptate and comparatively smaller conidia. The phialides of *Ph. sinense* mono- and polyphialidic; conidia obovate, sometimes cylindrical,

arranged in slimy heads, which was consistent with previous studies on *Phialoparvum* species (Giraldo et al. 2019; Giraldo and Crous 2019). *Ph. sinense* differs markedly from other *Phialoparvum* species by producing obovoid conidia and conspicuously longer phialides.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

The individual contributions are as follows: Zhi-Yuan Zhang and Gang Tao conceptualized the study, edited, and reviewed the manuscript. Heng Pan, Shuo-Qiu Tong, and Zeng Chen performed microscopical examinations of fungal specimens and wrote, edited, and reviewed the manuscript. Yun-Jie Wu and Yi Wang conducted phylogenetic studies, reviewed, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.

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